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Harmful Algae 6 (2007) 679–685
HARMFUL ALGAE
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Stable carbon isotope variations in surface bloom scum and subsurface seston among shallow eutrophic lakes

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Received 11 November 2006; received in revised form 30 November 2006; accepted 14 February 2007

Abstract

Carbon stable isotope analysis of surface bloom scum and subsurface seston samples was conducted in shallow eutrophic lakes in China during warm seasons from 2003 to 2004. $\delta^{13}C$ values of bloom scum were always higher (averaged 5‰) than those of seston in this study, and the possible reasons were attributed to (i) direct use of atmospheric CO_2 at the air–water interface, (ii) decrease in ^{13}C fractionation due to higher carbon fixation, (iii) active CO_2 transport, and/or (iv) HCO_3^- accumulation. Negative correlation between $\delta^{13}C_{scum} - \delta^{13}C_{seston}$ and pH in the test lakes indicated that phytoplankton at the subsurface water column increased isotopic enrichment under the carbon limitation along with the increase of pH, which might in turn decreased the differences in $\delta^{13}C$ between the subsurface seston and the surface scums. Significant positive correlations of seston $\delta^{13}C$ with total concentrations of nitrogen and phosphorus in water column suggested that the increase in $\delta^{13}C$ of seston with trophic state was depending on nutrient (N or P, or both) supply. Our study showed that $\delta^{13}C$ of phytoplankton was indicative of carbon utilization, primary productivity, and nutrient supply among the eutrophic lakes.

Keywords: Bloom scum; Eutrophic lakes; Seston; Stable carbon isotope

1. Introduction

Eutrophication is a complex process whereby fresh and marine water bodies become enriched by nutrients (e.g. phosphorus and nitrogen) from both external and internal sources (Harper, 1992; ECOHAB, 1997). It is considered as one of the most pressing environmental problems in both the developed and the developing countries (Ryding and Rast, 1989; Harper, 1992). The main effect of eutrophication is the imbalance in the base of food web that results in high levels of phytoplankton biomass, which can lead to algal blooms depending upon environmental factors, e.g. nutrients, light, temperature and water movement (Jacoby et al., 2000). Cyanobacterial blooms are the most frequently observed and widely studied algal blooms in freshwater and estuarine ecosystems throughout the world. Cyanobacterial blooms are often regarded as an indication of accelerating nutrient enrichment of aquatic ecosystems (Sellner et al., 2003). Cyanobacterial blooms may cause a variety of water quality problems, including dissolved oxygen depletion and subsequent death of aquatic animals, aesthetic nuisances (e.g. odors, massive surface scum, fish tainting), cyanotoxin production (e.g. microcystins), and unpalatable and possibly unsafe drinking water (Welch, 1992; Xie, 2006). Such problems can severely limit

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1568-9883/$ – see front matter
doi:10.1016/j.hal.2007.02.002

aquatic habitat, recreational activities, fisheries, and use of a water body for drinking water.

Stable carbon isotope analysis is a powerful tool to study carbon biogeochemical cycle in aquatic ecosystems (e.g. Kendall et al., 2001; Lehmann et al., 2004a,b). Stable carbon isotope ratio (δ^{13}C) of phytoplankton helps to gain substantial insights into sources and fate of carbon, primary productivity, and dissolved inorganic carbon (DIC) concentrations in the surface water (Kendall et al., 2001; Lehmann et al., 2004a,b). The δ^{13}C of phytoplankton in lacustrine systems varies over time and space (Gu et al., 1996; Lehmann et al., 2004b; Xu et al., 2005a), and these variations may be related to phytoplankton species composition, external nutrient input, and primary productivity, as well as sources and concentrations of DIC, their isotope signatures, and inherent isotope fractionations and kinetic mode of carbon fixation during photosynthesis (O'Leary, 1981; Rau et al., 1989; Falkowski, 1991; Grey and Jones, 2001). From the isotopic point of view, eutrophic lakes generally have a large potential to exhibit large spatial and temporal variations in δ^{13}C of phytoplankton due to dense algae blooms. Low DIC concentrations and high pH of lake water intensively affect the photosynthetic rate and imbalanced exchange between atmospheric CO_2 and aquatic carbon species (Zohary et al., 1994; Gu et al., 1996; Lehmann et al., 2004b; Xu et al., 2005a). For instance, phytoplankton fractionate against ^{13}C during carbon fixation, resulting in light isotopes in photosynthetic products and heavy isotopes in the DIC pool. However, when carbon demand is high and pool size is limited, phytoplankton incorporates all available C with little isotopic discrimination. Hence, during an algae bloom when primary production is increased and the pool size is reduced, δ^{13}C of phytoplankton may increase (Zohary et al., 1994; Gu et al., 1996; Xu et al., 2005a).

Many of the numerous shallow lakes in the middle and lower reaches of the Yangtze River area in subtropical China undergo severe eutrophication (Jin, 2003). Some of these lakes present cyanobacterial blooms (mainly composed of *Anabaena* and *Microcystis*) in warm seasons due to high nutrient levels and high water temperature, and surface scum is common in these eutrophic freshwaters (Wu et al., 2006). In this study, δ^{13}C of bloom scum ($\delta^{13}C_{scum}$) and seston ($\delta^{13}C_{seston}$) were investigated in 10 lakes, dominated by cyanobacterial bloom species, 9 of which were located in the middle and lower reaches of the Yangtze River, and 1 in Yunnan Plateau, in warm seasons between 2003 and 2004. The primary objectives were (i) to determine whether there are differences in δ^{13}C between bloom scums and seston, (ii) to evaluate relationships between environmental factors (e.g. nutrients, temperature, pH, etc.) and stable carbon isotopic compositions of scum and seston, and (iii) to discuss possible mechanisms underlying these observations in these shallow eutrophic lakes.

2. Material and methods

2.1. Study site

According to OECD (1982), the lakes were all in eutrophic and hypereutrophic states in terms of TP concentrations (Fig. 1). Phytoplankton biomass was high and consisted largely of cyanobacteria (mainly *Anabaena* and *Microcystis*). Aquatic macrophytes were scarce in these lakes, possibly because of nutrient stress, e.g. elevated NH_4^+, and light limitation (Cao et al., submitted to Marine and Freshwater Research). Lake Eastdongting is the only one connected with the Yangtze River throughout the year, and Lake Xingyun is the only exception which located in Yunnan Plateau, southwest China (Xu et al., 2005b). Limnological characteristics of these lakes were summarized in Table 1 (data from Wu et al., 2006; Xu et al., 2005a,b; Zhou et al., submitted to Ecological Engineering; Yang Hong, unpublished data).

2.2. Field sampling

One to four bloom scum samples were collected by scraping foam of phytoplankton from the surface of the

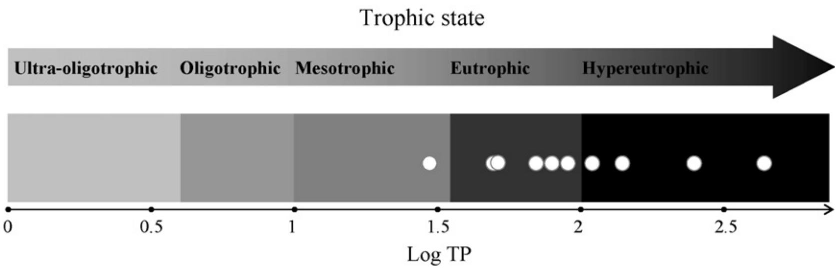

Fig. 1. Trophic states of the investigated lakes in this study classified according to total phosphorus concentration (OECD, 1982).

Table 1
Summary of limnological characteristics of the lakes in this study (data from Wu et al., 2006; Xu et al., 2005a,b; Zhou et al., submitted Ecological Engineering; Yang Hong, unpublished data)

Lake	Longitude	Latitude	Area (km^2)	depth (m)	Sechii (cm)	pH	Temperature (°C)	TN ($mg\ L^{-1}$)	TP ($mg\ L^{-1}$)	TC ($mg\ L^{-1}$)
Lake Chaohu	117°33′E	31°34′N	770	7.8	49	8.0	29.3	2.9	0.11	12.7
Lake Dianshan	120°58′E	31°10′N	64	2.2	33	8.5	30.5	1.5	0.25	30.5
Lake Eastdongting	113°02′E	29°15′N	1478	6.1	83	8.4	28.8	0.8	0.05	46.3
Lake Honghu	113°22′E	29°49′N	348	1.9	58	8.0	30.5	1.7	0.08	21.5
Lake Junshan	116°18′E	28°37′N	193	3.7	221	7.5	30.5	0.6	0.05	30.5
Lake Longgan	116°10′E	29°57′N	316	2.1	67	7.8	30.7	0.5	0.03	20.8
Lake Shijiu	118°53′E	31°30′N	210	2.3	113	7.8	26.0	0.7	0.07	14.8
Lake Taihu	120°10′E	31°26′N	2425	2.2	29	8.1	30.1	2.3	0.14	–
Lake Xingyun	102°47′E	24°20′N	35	5.3	18	8.8	23.8	3.2	0.44	–
Lake Yangcheng	120°49′E	31°25′N	119	1.6	78	8.3	30.0	0.6	0.09	29.5

Note: '–' indicate characteristics that were not available or not measured for some lakes used in this study.

water. Two to eight seston samples were collected by filtering subsurface lake water (0–0.5 m) near the sites where bloom scums were sampled through precombusted Whatman GF/C glass fiber filter having a 1.2 μm pore size. Water temperatures, depth, Sechii depth and pH were measured *in situ* with Water Quality Monitor (Model U-22, HORIBA Ltd.) and Sechii disk (Wu et al., 2006). For analysis of nutrients, water samples at each site were collected with Tygon water sampler, and then kept in 1 L acid-cleaned (1 M HCl) polyethylene bottles that were transported to the laboratory.

2.3. Laboratory analysis

In the laboratory, total nitrogen (TN) was determined by the alkaline potassium persulphate digestion-UV spectrophotometric method (Nydahl, 1978). Total phosphorus (TP) was determined by the ammonium molybdate method after potassium persulphate digestion (Prepas and Rigler, 1982). Bloom scum and filters of seston samples were acidified with superfluous 2 M HCl, and were then oven-dried at 60 °C to a constant weight and ground to a fine homogeneous powder using a mortar and pestle.

Stable carbon isotope ratios of scum and seston were analyzed with a Delta Plus (Finnigan) continuous flow isotope ratio mass spectrometer (CF-IRMS) directly coupled to an NC2500 elemental analyzer (Carlo Erba). The isotopic composition of samples was expressed as $\delta^{13}C$ notation using the following equation:

$$\delta^{13}\text{C}\ (‰) = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1\right) \times 10^3$$

where ‰ is parts per thousand and $^{13}C/^{12}C$ the atomic ratio of the number of atoms in the sample or standard, and δ is the proportion of heavy to light isotope in the sample, whereby higher δ values denote a greater proportion of the heavy isotope. The international standard is Vienna Pee Dee Belemnite (VPDB). International reference material is carbonatite (IAEA-NBS18), and on a daily basis, an internal working standard, urea ($\delta^{13}C = -49.44$‰), was used. More than 20% of the samples were analyzed two or more times and the standard errors of $\delta^{13}C$ replicate analyses were less than 0.3‰.

To examine correlations between $\delta^{13}C$ values of bloom scum and seston, and limnological parameters, STATISTIC for Windows statistical software (version 6.0) was used for the relative analyses.

3. Results

High TN and TP concentrations suggest that the investigated lakes were all nutrient-rich aquatic systems (Table 1; Fig. 1). Scum and seston were mixtures of phytoplankton, other minute organisms and organic detritus. During this study, phytoplankton production was extremely high, with dense water blooms of *Microcystis* and *Anabaena* observed, indicating that these samples mainly composed of phytoplankton. $\delta^{13}C$ of scum varied from −28.6‰ to −17.6‰, with an average of −23.4‰, and $\delta^{13}C$ of seston varied from −33.4‰ to −19.0‰, with an average of −28.7‰. $\delta^{13}C$ of both scum and seston varied among lakes (Table 2).

$\delta^{13}C$ values of the bloom scum were always higher (averaged 5‰) than those of seston in this study (*t*-test, $p < 0.01$, $n = 10$, Fig. 2). Weakly positive correlation was found between the isotopic ratios of scum and seston ($r = 0.60$, $p = 0.07$), which may partially have been attributed to the relatively small sample size. In the present study, no correlation were found between the

Table 2
Stable carbon isotopic values of average, standard deviation (S.D.), maximum (Max.) and minimum (Min.) and sample size of surface bloom scum and subsurface seston in the investigated lakes

	Surface bloom scum					Subsurface seston				
	Average	S.D.	Max.	Min.	*n*	Average	S.D.	Max.	Min.	*n*
Lake Chaohu[a]	−17.9	0.4	−17.6	−18.2	2	−26.2	1.8	−22.4	−31.7	74
Lake Dianshan	−27.9	1.1	−27.1	−28.6	2	−31.7	2.6	−29.9	−33.4	2
Lake Eastdongting	−28.5	0.0	−28.5	−28.5	2	−30.3	1.5	−29	−32.9	5
Lake Honghu	−23.4	1.4	−22.4	−24.4	2	−28.4	2.1	−25.7	−30.2	4
Lake Junshan	−20.0				1	−28.8	0.4	−28.4	−29.1	3
Lake Longgan	−22.7				1	−31.4	1.6	−30.2	−33.1	3
Lake Shijiu	−24.2				1	−30.4	1.1	−29.7	−31.2	2
Lake Taihu[b]	−23.2	1.2	−24.0	−22.4	2	−29.4	1.6	−25.1	−32.9	56
Lake Xingyun	−20.4	0.6	−19.6	−20.9	4	−21.2	1.4	−19	−23.3	8
Lake Yangcheng	−26.2				1	−28.9	2.3	−25.6	−30.1	4

[a] $\delta^{13}C$ values of seston from Lake Chaohu were cited from Xu et al. (2005a,b).
[b] $\delta^{13}C$ values of seston from Lake Taihu were provided by Zhou (submitted to Ecological Engineering).

measured limnological parameters and $\delta^{13}C$ values of scum, while the increase of seston $\delta^{13}C$ values was accompanied by the increase of total nitrogen and phosphorus concentrations ($r = 0.76$, $p < 0.05$ for TN, $r = 0.70$, $p < 0.05$ for TP, Fig. 3). Another striking observation was that the difference between $\delta^{13}C_{scum}$ and $\delta^{13}C_{seston}$ showed significantly negative correlation with pH in these lakes ($r = -0.88$, $p < 0.001$, $n = 10$, Fig. 4).

4. Discussion

$\delta^{13}C$ values of scum and seston, which was mainly comprised of *Microcystis* spp. and *Anabaena* spp., fell within the published range of values from freshwater plankton (Kendall et al., 2001; Vuorio et al., 2006). Generally, the $\delta^{13}C$ value of phytoplankton is affected by DIC availability, its isotopic signature, and phytoplankton physiology (Paterson and Whitfield, 1997). Numerous studies indicate that the $\delta^{13}C$ variability of phytoplankton can be largely attributed to carbon demand and DIC availability in water (Takahashi et al., 1990; Gu and Schelske, 1996). Large isotope fractionation by phytoplankton can be expected when there is abundant DIC and/or when the growth rate

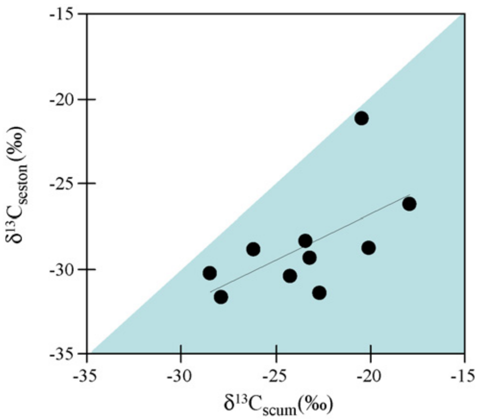

Fig. 2. Relationship between $\delta^{13}C$ values of subsurface seston and surface bloom scum. In the shaded region, $\delta^{13}C$ values of surface bloom scum are greater than or equal to $\delta^{13}C$ values of subsurface seston. $\delta^{13}C$ of subsurface seston was weakly correlated $\delta^{13}C$ of surface scum ($r = 0.60$, $p = 0.07$, $n = 10$).

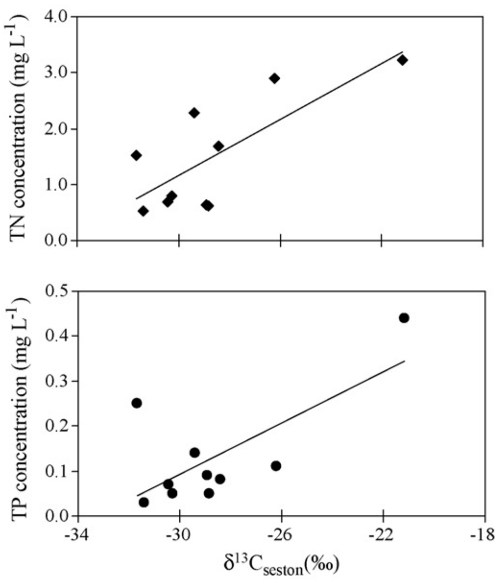

Fig. 3. Relationship between $\delta^{13}C$ values of subsurface seston and total nitrogen and phosphorus concentrations of lake water. Significantly positive correlations were found between $\delta^{13}C$ values of subsurface seston and total nitrogen ($r = 0.76$, $p < 0.05$, $n = 10$) and phosphorus concentrations ($r = 0.70$, $p < 0.05$, $n = 10$) of lake water.

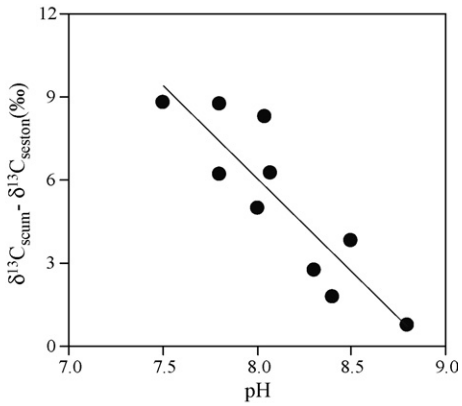

Fig. 4. Relationship between the values of $\delta^{13}C_{scum} - \delta^{13}C_{seston}$ and pH in this study. Dashed line is the trend line that best describes the relationship with a linear equation of $Y = 59.6 - 6.7X$ ($r = -0.88$, $p < 0.001$, $n = 10$).

is low (Lehmann et al., 2004a,b; Gu et al., 2006). In eutrophic lakes, phytoplankton rapidly assimilate ^{12}C during periods of active photosynthesis, leaving the DIC pool enriched with ^{13}C during periods of high growth, and subsequent carbon fixation in surface water will use the heavy DIC and lead to ^{13}C enrichment in organic matter (Lehmann et al., 2004b; Xu et al., 2005a; Gu et al., 2006). Furthermore, decreased ambient dissolved CO_2 concentration may trigger active CO_2 transport or taking up HCO_3^- by phytoplankton which subsequently in the cell is dehydrated to CO_2 by carbonic anhydrase immediately (carbon concentration mechanism, CCM) (Takahashi et al., 1990; Fogel et al., 1992; Raven, 2003). These processes further enrich phytoplankton with ^{13}C. A number of studies have demonstrated that many plants were capable of switching to a CCM in response to increasing HCO_3^- concentrations and dissolved CO_2 limitation (Fogel et al., 1992; Popp et al., 1998; Matsuda et al., 2001; Rau et al., 2001; Raven, 2003). Another mechanism that directly contributes to enrichment of DIC $\delta^{13}C$ in these shallow eutrophic lakes may be the bacterial degradation of organic matter and hence recycling of carbon. For example, the forms of anaerobic respiration, e.g. methanogenesis, in the sediments produce ^{13}C-enriched CO_2 and ^{13}C-depleted CH_4 (Gu and Schelske, 2004), and the ^{13}C-depleted carbon is lost from the lake water column by CH_4 ebullition living the isotopically heavy CO_2 in the water column (Wachniew and Różański, 1997; Gu and Schelske, 2004).

Cyanobacterial surface scums are common in eutrophic freshwater systems (Reynolds and Walsby, 1975; Paerl, 1996). It has been proposed that surface scum formation is an ecological response to carbon limitation in surface water (Paerl and Ustach, 1982). In the present study, carbon isotopic signatures differ significantly between scum and seston in the shallow eutrophic lakes, suggesting different carbon acquisition mechanisms and/or carbon limiting conditions between phytoplankton communities in surface and subsurface layers of lake water. During our investigation, near-surface pH values were typically higher than 7 in all the studied lakes, which is commonly observed in eutrophic water bodies (Takahashi et al., 1990; Gu and Alexander, 1996); at such a pH level, dissolved CO_2 concentrations was expected to be low and HCO_3^- was the major form of DIC (Takahashi et al., 1990; Fogel et al., 1992; Gu et al., 2006; Myrbo and Shapley, 2006). Phytoplankton communities in the surface bloom scum appeared more severely carbon limited than phytoplankton present in subsurface layers because of their high rates of photosynthesis. This could result more reduced discrimination against $^{13}CO_2$ or a shift toward a CCM during photosynthesis. An alternative explanation for highly enriched $\delta^{13}C$ values in scum with regard to seston is that phytoplankton in the scum directly utilize atmospheric CO_2 at the air–water interface under calm conditions (Paerl, 1979; Paerl and Ustach, 1982; Gu and Alexander, 1996). If surface scums ceased carbon fixation, it would presumably have similar $\delta^{13}C$ to subsurface seston. Because isotope fractionation during the decay of organic matter is small, it is unlikely that the large ^{13}C enrichment of surface scums observed here was caused by isotope fractionation during scum decomposition (Gu and Alexander, 1996). Since isotope fractionation between the scums and subsurface seston relative to their respective carbon sources were similar and atmospheric CO_2 typically has high $\delta^{13}C$ compared with DIC, the scums must have fixed atmospheric CO_2 directly to result in such a large isotope enrichment (Gu and Alexander, 1996). The large range (0.7–8.8‰) of differences in $\delta^{13}C$ between the surface scums and subsurface seston probably reflected the variation in the proportion of atmospheric CO_2 the scums fixed, which might in turn have depended on the time they remained at the water surface (Gu and Alexander, 1996). We also observed a significantly negative correlation between $\delta^{13}C_{scum} - \delta^{13}C_{seston}$ and pH value, with neither $\delta^{13}C_{scum}$ nor $\delta^{13}C_{seston}$ being statistically correlated with pH. It was reasonable to assume that, under the increased carbon limitation along with the increase of pH, significant utilization of ^{13}C-enriched DIC by the cyanobacterial populations at the subsurface water column might have taken place to result in a large isotope enrichment of the subsurface seston, which might in turn decrease the differences in $\delta^{13}C$ between the subsurface seston and the surface scums.

Significant positive correlations of seston $\delta^{13}C$ with total concentrations of nitrogen and phosphorus were observed in these lakes, presumably reflecting the influence of external nutrient input on primary productivity within these systems. The influence of these chemical variables in eutrophic waters is linked with multiple variables of different impacts, through which result in similar isotopic signatures. For example, increasing nutrient input increases primary productivity, which elevates pH value and decreases aquatic CO_2 concentration. The depletion of dissolved CO_2 further leads to little isotopic discrimination and enriched ^{13}C of phytoplankton, and simultaneously facilitates assimilation of HCO_3^-, which can lead to high $\delta^{13}C$ signatures similar to those induced by pure diffusion limitation. Supplies of nitrogen or phosphorus or both often limit phytoplankton growth (Klausmeier et al., 2004). In eutrophic lakes, an increase in $\delta^{13}C$ may be expected when greater N or P availability stimulates algal carbon fixation. Decreases in $\delta^{13}C$ fractionation by terrestrial and aquatic plants with increases in inorganic nitrogen supply have been documented (Bender and Berge, 1979; Raven and Farquhar, 1990; Jenkinson et al., 1995; Gu et al., 1999). $\delta^{13}C$ of sediment organic carbon increased in response to historic increases in P loading and then decreased in response to reduced P loading (Schelske and Hodell, 1991, 1995). Significant correlations between TN and TP of the overlying water and sediment $\delta^{13}C$ and were attributed to that primary productivity increased with TN or TP or both in Florida lakes (Gu et al., 1996). These studies supported our finding that increase in $\delta^{13}C$ of seston with trophic state was depending on nutrient (N or P, or both) supply.

5. Conclusion

$\delta^{13}C$ of scum and seston in these shallow eutrophic lakes can provide important information about ambient environmental conditions and carbon biogeochemical cycling among the systems. These observations could be helpful to infer changes in primary productivity and water quality in aquatic ecosystems affected by anthropogenic activities. Further study focusing on the isotopic composition of DIC, planktonic assemblages and corresponding isotopic compositions, and the relationships with water quality variables, will help improve our understanding of carbon cycling in these ecosystems.

Acknowledgements

Drs. Binhe Gu, Thomas Dreschel and two anonymous reviewers provided valuable comments on an early version of this manuscript. We would like to express our thanks to Drs. Shikai Wu, Sixin Li, Hong Yang, and Qiong Zhou for providing scum and seston samples, and unpublished limnological parameters. This study was supported by key projects of Chinese Academy of Sciences (Grant No. KZCX1-SW-13 and KSCX2-SW-129), a Chinese Nature Science Foundation Project (Grant No. 30600089), and by a fund of the field stations from Chinese Academy of Sciences with the title "Constructions of the food webs in Lakes Donghu, Taihu and Chaohu using $\delta^{13}C$ and $\delta^{15}N$".[SS]

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